

- conclup*
- a) mixing at least one labeled random primer at least 4 nucleotides in length having at least one first label and optionally at least one second random primer at least 4 nucleotides in length having at least one second label, with a sample nucleic acid,
 - b) adding at least one nucleic acid ligase and at least one nucleic acid polymerase,
 - c) adding at least one nucleotide triphosphate,
 - d) incubating the mixture of step c), under conditions which allow said at least one nucleic acid ligase and at least one nucleic acid polymerase to be active,
 - e) quantitating the amount of nucleic acid in said sample by detecting and/or quantitating the amount of said at least one first label or the amount of said at least one second label. --

REMARKS

Reconsideration and allowance of the subject application are respectfully requested.

The assay method of the subject invention is surprising in its ability to quantitate total DNA in a variety of samples that can vary considerably in the complexity of the mixture, in the types of sequences present and in the length of the sequences. One skilled in the art would have expected that the generated signal would vary considerably for different samples: i) the affinity of the nucleic acid duplexes that form on a particular sequence of sample nucleic acid should vary considerably with the nucleic acid sequence (these variations in affinity would be especially important for the short primer sequences that are preferred); ii) the activity of the polymerases and ligases would also be expected to depend on the nucleic acid sequence being acted on; iii)

the length of the enzymatic reaction products (and the assay signals derived from them) would be expected to vary considerably depending on the length of the nucleic acid sample and iv) the affinities of the primers and the activities of the enzymes would also be expected to depend considerably on the presence of secondary structure in the nucleic acid sample. The Examples in the specification clearly show the ability of the invention to accurately quantitate nucleic acids from different complex samples despite all these potential problems.

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Claims 1-37 stand rejected under 35 USC 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Reconsideration is requested.

The claims have been amended to obviate this rejection.

Claims 1-3, 6-12, 14-25, 28-32 and 34-37 stand rejected under 35 USC 103(a) as being unpatentable over Hartley in view of Eberle et al. Reconsideration is requested.

The methods of the invention measure the total amount of nucleic acid. Hartley uses random primers as generic reagents for amplifying nucleic acids but only describes assays for specific DNA sequences. In Hartley, the sample DNA is either a pure defined sequence (examples 1-2) or the desired sequence is prepurified by a literature method (example 3) or by using a specific capture probe on magnetic beads (examples 4-6). In all the examples except example 4, an additional layer of specificity is added through the detection of the amplified products on "dot blots" using a radioactive probe specific for the sequence being analyzed.

Eberle does not describe methods for quantitating nucleic acids. Eberle describes methods for quantitating polymerases. As such, the reference uses defined nucleic acids in

known concentrations as reagents. It does not teach methods for quantitating nucleic acids of unknown sequence and length in possibly complex mixtures of nucleic acids. Eberle does not teach that the signal obtained from a polymerase assay would be independent of the sequence, size or complexity of the nucleic acid reagent used as a template.

Hartley and Eberle are improperly combined by the Examiner. Hartley uses random primers for amplifying specific sequences of DNA. Eberle measures polymerases by incorporating detectable and binding NTPs in amplified nucleic acid. Despite the disclosure in Hartley of primers with binding groups, there is no suggestion in either reference that the combination of a binding group on the primer and a detectable group on the amplified product would either work or be useful.

Claims 4, 5, 13, 26, 27 and 33 stand rejected under 35 USC 103(a) as being unpatentable over Hartley in view of Wu et al and Respass. Reconsideration is requested.

Hartley is discussed above. The Respass patent and the Wu article are both directed towards methods for amplifying a detecting specific nucleic acid sequences. The Examiner states that the amplified products have binding species because they hybridize to bound probes. The specification makes it clear that the binding species is a moiety linked to a nucleic acid and not the nucleic acid itself. By using the nucleic acid itself as the binding species, the assay can only measure DNA of a known sequence. Applicants would also disagree with the statement that knowledge of the use of random primers for polymerases would provide one with a reasonable expectation with a different class of enzymes, ligases.

In view of the above, Applicants respectfully submit that all claims now pending herein fully and patentably define the present invention over the applied art of record. As such, early

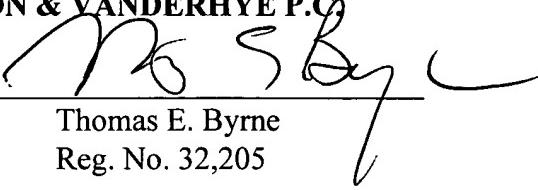
Jeffrey A. Heroux, et al
Serial No. 09/023,483

receipt of the Official Notice of Allowance is awaited.

Should any small matters remain outstanding, the Examiner is encouraged to telephone Applicants' undersigned attorney so that same can be resolved without the necessity of an additional Action and response thereto.

Respectfully submitted,
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